

PHENOTYPIC MICROARRAY ANALYSIS FOR PHENOMICS AND PATHWAY ANALYSES IN ANAEROBES

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RESEARCH OBJECTIVES

Phenotypic Microarray™ analysis is a recently developed analytical tool to determine the phenotype of an organism. This technique can be useful in understanding the growth changes of an organism when changing medium, temperature, or adding a stressor, or when testing mutant strains. The tool, which is commercially available from Biolog™ (Hayward, CA), consists of an array of 20 plates. The first eight plates test a variety of metabolic agents, including electron donors, acceptors, and amino acids. Plates 9 and 10 cover pH and osmotic stressors, while plates 11–20 contain a variety of inhibitors, including toxic agents and antibiotics. In total, the plates simultaneously test nearly 2,000 independent conditions on a single bacteria culture.



Figure 1. Growth of *Desulfovibrio vulgaris* in phenotypic microarray plate 10 (PM10). This plate measures the effect of pH on growth. Each plot represents growth in a different pH condition, the horizontal axis representing time (7 days), the vertical axis representing increase in turbidity, which is correlated with growth.

APPROACH

Techniques have been developed at Berkeley Lab for using these plates under anaerobic condition, to enable culturing of an obligate anaerobe, *Desulfovibrio vulgaris*. To accomplish this task, plates were set up in an anaerobic chamber and heat-sealed in polyethylene bags containing an anaerobic sachet. This technique permitted maintenance of anaerobic conditions

in the plates for up to a week. Growth of the cells was measured by the increase in turbidity of the cells, which was correlated with both optical densities at 600 nm and total cell counts. Preconditioning of the cells and specialized media preparation are required for the different types of plates to obtain a valid phenotype.

ACCOMPLISHMENTS

The plates have been successfully used to characterize the phenotype of the *Desulfovibrio vulgaris* American Type Culture Collection (ATCC) strain (see Figure 1). The plates are currently being applied to mutant strains to provide rapid screening of mutant phenotypic changes needed for rapid pathway analyses and modeling. Several method-development obstacles have been overcome, including optimization of the plate-sealing technique, density of the inoculated organisms, and false positive and negative results from either excess or deficient inoculum.

SIGNIFICANCE OF FINDINGS

This technique will allow the production of high-volume and high-quality data related to the effect of targeted mutations and environmental stressors on bacterial cultures. This array, which will provide a unique tool for the study of bacterial stress and survival, is currently being applied to metal-reducing bacteria in soil.

RELATED PUBLICATION

S. Borglin, T. Hazen, J. Carlson, J. Wall, D. Joyner. Phenotypic microarray analysis of *Desulfovibrio vulgaris*. ASM General Meeting, Atlanta, Georgia, June 8, 2005.

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